SOME PROTEIN-RICH INFANT FOODS BASED ON RICE

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ABSTRACT

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by Ruth Wen Chen

Several enriched foods, taking rice flour as the basic ingredient (75-80%), were designed and tested for food efficiency and protein efficiency ratio (PER) at a protein level of 15% total protein before and after storage at 100% relative humidity and 95°F. for 145 days, and, fresh, at 10% total protein, by feeding these diets to male weanling rats.

The proposed diets are dry flour-like products prepared with water in porridge form, similar to rice flour in appearance, and contain in addition to a basic supplement of yeast, sweet potato, $CaCO_3$ and salt, various amounts of codfish flour and soybean flour. Eaten in amounts of 250-300 g per day by one year old (9 kg) infants, they will supply all the basic nutrients except vitamin C and vitamin D.

These proposed diets, as judged by PER and food efficiency, are better than either soybean or skim milk. A proper combination of vegetable and animal protein can give the same benefit as an animal protein, such as codfish, alone.

The proposed diets after storage were slightly darker, lost up to 50% β-carotene, and developed off-flavors and odors which were, depending on the particular diet, marginally acceptable or unacceptable, although they still gave good PER values.

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By

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INTRODUCTION

Malnutrition is the number one world health problem. People have a less adequate diet today than they did at the beginning of this century (Brown, 1963). Mortality rates are twenty to forty times higher for infants and pre-school children in less developed areas than in more favored countries (FAO, 1963a). In 1963, according to an FAO survey (Brown, 1963), 20% of the people in developing countries were undernourished and 60% were malnourished; that means 10-15% of the world population is undernourished and an additional 50% are suffering from hunger, malnutrition or both (Belden _et al._, 1964). United Nations officials estimated that almost one-third of the children of Latin America, Asia, and the Middle East will die this year from hunger and diseases associated with malnutrition, and more than one-fifth from tainted water (Ross, 1966).

In most types of malnutrition, the children's growth is retarded (Jackson, 1966); they have low resistance to disease (Wills and Waterlow, 1958; Scrimshaw _et al._, 1959), poor psychomotor development, and high mortality rates, particularly children less than five years of age (FAO, 1963a). With outside capital and technical assistance, many less developed countries have attempted to increase food production, but thus far they have not corrected malnutrition. The food available per person is still decreasing despite crop increases because the population has increased much faster than food production (FAO, 1963a).

Most Asian countries are politically unstable and are still developing economically (FAO, 1963b). The Asian income is so low that the people live almost entirely on vegetables and cereals (WHO, 1965). Tradition says that rice gruel alone will make babies healthy and strong. Moreover, from the economic standpoint, the parents cannot afford to feed babies animal protein (Do-Quang-Oanh, 1963). Typically, 80% of the calories in the infant diet is from rice and 20% from sugar, which means about 13 g protein (protein score 72) and 900 calories per day. Consequently, the highest incidence of protein malnutrition in children is in these areas, as reported by WHO (1965).

Schemes to solve the nutrition problems in the developing countries at a price within the reach of the population are needed. Supplementation with high quality animal proteins--meat, egg, fish, milk and dairy products--is not now possible because supplies are inadequate and/or too costly (Belden et al., 1964). Attention has been focused on making well balanced vegetable mixtures or on adding small amounts of animal proteins to the existing diets.

Any food designed for developing countries must conform to certain principles. Milner (1962) suggests, among others, the following properties: capable of local production; within the economic means of the population; easily transportable and capable of non-refrigerated storage in the local climate; and acceptable to the population.

Low quality protein food can be improved by adding protein concentrates or supplements, as defined by the FAO-WHO Protein Advisory Group, to the basic rice diet. The purpose of the present work is to design some protein-rich foods, based on rice, which are low in cost, easily kept and transported, acceptable, and which have a nutritive value sufficient to prevent malnutrition of infants in rice-eating countries.

LITERATURE REVIEW

Nutrition Requirements for Infants

Nutritional requirements for infants are similar to those for adults in many respects, but different in certain important respects. These differences will be noted where information is available on the subject.

Protein and Amino Acids

The quantity of protein required depends upon the actual amino acid pattern of the diet and the extent to which protein is spared by carbohydrate and fat. A recent approach to protein requirements was reported by the WHO-FAO Joint Expert Group in 1965 (WHO, 1965). They concluded that 6- to 9-month old infants require 1.5 g protein/kg body weight from breast milk, cow's milk, or from any source of equal quality; 9- to 12-month old children require 1.2 g protein/kg body weight.

Protein quality depends upon the amino acids of the protein, primarily the essential amino acids, through non-essential amino acids may be involved (Snyderman et al., 1962). Although the FAO Committee on Protein Requirement (FAO, 1957) did give an ideal amino acid pattern for adults and children, the Joint Expert Group (WHO, 1965) simply suggested human milk and whole egg patterns as guides for protein quality evaluation. This suggested pattern was not given specifically for children. However, a committee on amino acids has worked on infant amino acid requirements (NAS/NRC, 1959). Two of these patterns are given in Table 1. Histidine is considered essential for the young infant (Snyderman et al., 1963) but it is not considered in other patterns except the NAS/NRC (1959) recommended pattern. Cysteine and tyrosine, two non-essential amino acids available to spare phenylalanine and methionine (Holt) and Snyderman, 1965), are not considered in NAS/NRC (1959) pattern. It is hard to determine which pattern, if any, is the best because experiments on amino acid requirements give results which vary with experimental conditions. Holt and Snyderman (1965) concluded only that with more knowledge of amino acid interrelations and assistance from a computer could amino acid patterns of diets be accurately evaluated. Better clinical and chemical criteria of protein malnutrition also need to be established. The design of adequate diets from "book" values depends, of course, on a well-established standard.

Vitamins

Bitamin B_1 or thiamine.--Thiamine deficiency disease, beriberi, is prevalent in areas where polished white rice is the staple food. It causes the death of an unknown number of infants and children in Burma, Thailand, the Philippines, and other parts of Asia (NAS/NRC, 1966). The

TABLE 1.--Comparison of two recommended amino acid patterns with those of several foods (mg/g total essential amino acid).

Amino acid	Recommended		Actual[d]		
	FAO, 1957[a]	NAS/NRC,1959[b,c]	Cow's milk	Human milk	Hen's egg
Histidine	-	44	-	-	-
Isoleucine	134	124	127	132	129
Leucine	152	206	196	183	172
Lysine	134	144	155	128	125
Total aromatic	178		197	208	195
Phenylalanine	89	124	97	95	114
Tyrosine	89		100	113	81
Total sulfur-containing	133		65	88	107
Cysteine	62		17	43	46
Methionine	71	117	48	44	61
Threonine	89	82	91	93	99
Tryptophan	45	30	28	34	31
Valine	134	128	137	134	141

[a]For adults and infants.

[b]For infants only.

[c]Converted to mg amino acid/g total essential amino acids from the original data given as mg amino acid/kg body weight.

[d]The WHO (1965) human milk values appear to be incorrect in that table and have been readjusted here.

approximate daily requirement is 0.2 mg of thiamine per 1000 calories (Holt et al., 1949) but 0.4 mg per 1000 calories is recommended (NAS/NRC, 1964). Food preparation, heat, acidity, and moisture will influence the degree of thiamine destruction. As temperature and moisture increase, more thiamine is destroyed (Heinz, 1963).

Niacin.--Fatigue, irritability, and weight loss are early symptoms of niacin deficiency, followed by dementia, diarrhea, dermatitis and death (Heinz, 1962). The requirement for infants is 6 mg equivalent niacin per day (NAS/NRC, 1964). In vivo, 60 mg tryptophan can convert to 1 mg niacin (Heinz, 1963). Although rice is a poor source of niacin, examples of niacin deficiency in rice-eating areas have not been reported, but probably exist.

Vitamin B_2 or riboflavin.--Deficiency of vitamin B_2 causes corneal vascularization, ulceration cataract, and abnormal pigmentation of the iris (Heinz, 1963). The requirement of riboflavin for infants is 0.4 mg per day (Snyderman et al., 1949).

Vitamin B_6.--The most prominent symptom of vitamin B_6 deficiency in infancy is convulsive seizures, hemosiderosis and hypochromic anemia might also be caused by insufficient vitamin B_6 (Heinz, 1963). The requirement of pyridoxine for infants is 0.5 mg per day (Tenth M and R Pediatric Research Conference, 1953).

Folic acid and B_{12}.--Folic acid and B_{12}, which can be synthesized in the large intestine, are not a problem in the infant's diet (Holt and Snyderman, 1964).

Other vitamin B factors.--Pathothenic acid, biotin, and inositol are not demonstrated as normal dietary essentials (Holt and Snyderman, 1964).

Vitamin C or ascorbic acid.--In vitamin C deficiency, scurvy occurs mostly between ages of 5 and 24 months, with a peak incidence at 5 to 11 months (Woodruff, 1964). In a well-nourished population, the amount of ascorbic acid received from the mother is apparently sufficient to prevent scurvy up to about 5 months of life. But artificially fed infants or infants past the age of breast-feeding need a certain amount of ascorbic acid, about 25 mg/day. Ascorbic acid is stable in dry form but is easily oxidized in aqueous solution in the presence of air. The rate of oxidation is greatly increased by a trace of copper or by high pH. Processing methods and storage generally reduce the amount of vitamin C in a food. Citrus fruits and their juices and raw or minimally cooked vegetables are good sources of vitamin C (Holt and Snyderman, 1964).

Vitamin A.--Night blindness, xerophthalmia, and kerotomalcia are symptoms of vitamin A deficiency. It is prevalent throughout Asia, the Middle East, parts of Africa, and Latin America. The mortality rate among malnourished children with xerophthalmia is very high because of the many other health factors adversely affected by vitamin A deficiency. The incidence of xerophthalmia is increasing (McLaren, 1966) and fortification of the diet with vitamin A is required in these developing areas. The vitamin A requirement for infants is not accurately known yet, but 1500 I.U./day or 0.9 mg β-carotene/day is definitely protective (NAS/NRC, 1964; Heinz, 1959).

Vitamin D.--Infants require 400 I.U. vitamin D/day. Since 7-dehydrocholesterol (vitamin D_3) can be converted by provitamin D in the skin under ultraviolet light (Heinz, 1963), the requirement for this vitamin can be met by adequate exposure of the body to sunlight.

Vitamin E and vitamin K.--The requirement of vitamin E for normal infants has not been established (Holt and Snyderman, 1964). Vitamin K deficiency in infants occurs only in clinical conditions from malabsorption from the intestine or from liver disease (Holt and Snyderman, 1964).

Minerals

Calcium.--The requirement of calcium increases in the stage of skeletal growth from 250 mg/day to 700 mg/day in the first year (NAS/NRC, 1964). Milk-free diets need to be fortified with calcium (Holt and Snyderman, 1964).

Iron.--Iron deficiency anemia, which may or may not be associated with protein-calorie malnutrition, is widespread throughout the developing areas (Finch, 1966). The requirement of iron for infants is 1 mg/kg body weight (Moore, 1964). Iron deficiency anemia is observed more often in breast-fed infants than in artificically fed infants since milk is a poor source of iron (Holt and Snyderman, 1964).

Sodium.--Sodium deficiency could be a problem in children fed cereal only since cereal grains are a poor source of sodium. Infants require 120 mg/day (Holt and Snyderman, 1964).

Other minerals.--Infants require per day 420 mg potassium, 30 mg manganese, 330 mg chloride, and 200 mg phosphorous to maintain health and to promote growth. Usually infants can get enough intake of these minerals from most diets (Holt and Snyderman, 1964).

Iodine deficiency is prevalent in mountain areas of the world. In many villages, most people have endemic goiter. Cretinism and dwarfism are the symptoms of iodine deficiency in children. The requirements of iodine for infants is not known yet, but the addition of iodine to the diet is required to cure or prevent this deficiency where it exists (Follis, 1966).

Water

Infants are peculiarly susceptible to a lack of water. Water loss through the kidneys and skin is much greater than that of adults because of a larger surface to volume ratio (Holt and Snyderman, 1964). Water requirements are influenced by weather; for instance, hot weather increases the water requirement, but not that of other nutrients (Norman and Pratt, 1958). In a temperate climate, infants require 150 c.c. per kilogram body weight, but under subtropical or tropical conditions they may need 175 c.c./kg body weight or more (Holt and Snyderman, 1964).

Fats

Some evidence is reported that infants require a small quantity of polyunsaturated fatty acids (Wiese, Hansen, and Adam, 1958; Adam, Hansen, and Wiese, 1958; Hansen *et al.*, 1958), but what the optimum intake is has not been resolved (Holt and Snyderman, 1964). However, in the absence of further data, it seems safe and possibly desirable to include some unsaturated fatty acids in infant diets.

Energy Requirements

Energy is required for basal metabolism, physical exercise, and the specific dynamic effect of food. The daily energy need for infants is 120 cal/kg body weight (Hytten and MacQueen, 1954) or as Holt and Snyderman (1964) suggested, 70 cal/kg body weight for placid infants and 130 cal/kg body weight for crying babies.

In diet design, there are a number of nutritional factors involved, some more critical than others. If a child eats the right amount of rice to meet his calorie requirement, then he may suffer from thiamine, niacin, riboflavin, vitamin C, vitamin D, vitamin A, and protein deficiency. Present knowledge is good enough to design more adequate diets for infants.

Schemes to Prevent or Cure Malnutrition

Up to the time of weaning, babies are adequately nourished by their mothers' milk (Gopalan and Belavady, 1961). But in the weaning and after weaning periods, the child is not strong enough to fend for himself in the family circle; protein-calorie deficiency occurs frequently (WHO, 1965). Shortages of milk and other animal proteins, where they occur, and the likelihood that these shortages will persist, has focused attention on vegetable proteins which are relatively inexpensive (Teply and György, 1962). It was reported by several groups that the mixture of a relatively small proportion of skim milk powder (Nicholo and Phillips, 1961; Gopalan, 1961) or of fish flour (Sure, 1957; Seneal, 1961; Graham, Baertle, and Cordans, 1962, 1965) be combined with vegetable proteins to provide adequate protein for infants. There are many schemes and projects, all at different stages of progress, for different areas of the world. The following is a summary of some of these programs.

Asia

In Indonesia, the incidence of vitamin A deficiency is high. In 1963, red-palm oil was distributed, in one village, to mothers to be fed to children for prevention of xerophthalmia, and the incidence of the disease dropped significantly (György, 1966). The United Nations Chidren's Fund (UNICEF) provided assistance in producing an infant diet called Saridle, a product based on whole dehulled beans as the sole protein source, originally based on soy extract. The standardization of this product is still not well established (FAO/UNICEF, 1965). The formula consisting of skim milk : sugar : mixture of oils (coconut, peanut, and red-palm oil) in the proportions 45 : 50 : 30 is under study (György, 1966).

In India, the Central Food Technological Research Institute, CFTRI, at Mysore, has produced multipurpose foods for infants and pre-school children. These foods have been fortified with vitamins A, D, and riboflavin, and calcium and iron salts. They can be classified into the following groups: (a) High-protein foods (40% protein or more), used as dietary supplements for pre-school children, based on blends of oil-seed meals, legumes, skim milk powder and fish flour; (b) Cereals and biscuits (18 to 26% protein), used as supplements or complete foods; (c) High-protein foods (60% protein or more), used as a supplement in the treatment of protein malnutrition in children, based on peanut-protein isolate, chickpea, and skim milk powder; (d) A dried milk substitute, used for infants, is based on soybean, ground nut, and buffalo milk, and when used for weaning infants, is based on micro-atomized oil-seed meal (Subrahmanya <u>et al.</u>, 1957;

Venkatachalam, and Srikantic, 1960; Bhatia and Swaminathan, 1963; Doraiswamy et al., 1963; Parpia, 1966).

In Lebanon, evaluation of protein in sweet almond, which is used for infant feeding, was done by Cowan et al. (1963). Methionine, threonine, and tryptophan are three limiting amino acids. The supplementation of sesame, a good source of lysine and threonine, and chickpea, a good source of lysine and threonine, might improve the quality of almond as Cowan suggested. But he believed that within the existing dietary pattern in the Middle East, such a food would not be accepted.

In the Philippines, the infant mortality rate in 1962 was 2.7 times higher than in the United States; the death rate in children aged 1 to 4 years was forty-five times higher (Department of Health, 1962; U. N., 1963). In 1962, the Department of Health of the Philippines reported that beriberi was one of the leading causes of infant death (Department of Health, 1962). In 1948, the National Rice and Corn Cooperation, NARIC, manufactured and distributed to Bataan province a premix which was added to rice and which contained thiamine, niacin, and iron. The mortality from beriberi was reduced to zero after two years of using enriched rice in that province. The price of this enriched rice to the consumer is 2% higher (Furter et al., 1946). The Philippine experience is a good but discouraging example of a food which will solve the nutritional problems, but has not, for various reasons, been widely used. As Williams (1964) said, ". . . the extent to which rice enrichment is practiced in the Philippines is virtually negligible."

In Taiwan, 4-month old infants from lower middle class families were fed a test diet based on soybean, to which rice flour, sugar, minerals, a vitamin mixture, and soybean oil were added. It was found, based on a 12-month feeding test, that these full-fat soy products were practical and satisfactory dietary alternatives to animal milk provided that they were fed at levels which are more than critical in proteins and calories (DeMaeyer, 1965).

Africa

For infants in Uganda, the British have tried a fortified biscuit made of peanut, cereal flour, and sucrose (FAO/UNICEF, 1965).

In Senegal, the French have tested a millet, peanut, and fish mixture for infants. It was hoped that by 1965 a precooked weaning food, couscous, made from grain sorghum, peanut protein concentrate, skim milk powder, and sugar would be introduced on the market (Teply, 1964).

In Ethiopia, it is planned that the Children's Nutrition Unit in Addis Ababa will provide basic facts and outline general policy for infant feeding. A basic health service would then be the institution responsible for an ". . . integrated combat against malnutrition" (Mannheimer, 1966).

In South Africa, ProNutro, a supplement containing 21.5% protein, 265 cal/100 g, has been produced in Durban by a commercial firm. The initial product, Incumbe, was made in 1938, and since then has been much modified and improved to the present product, ProNutro. The final formula consists of skim milk, whole soybean and peanuts, fish protein concentrate, food yeast, wheat germ, whey powder, bone meal with addition of iron, iodized salt, thiamine, riboflavin, and niacin; sugar is added for palatability. ProNutro was clinically tested successfully in the prevention of kwashiorkor and other severe states of under or malnutrition in pre-school children (Simson and Mann, 1961) and other age groups. Two products are available; standard flaked ProNutro is 14 U. S. cents per pound, soup powder is 15 U. S. cents per pound (Belden _et al._, 1964).

Central and South America

In Central America, Incaparina, a dietary supplement for pre-school children, was developed at the Institute of Nutrition of Central America and Panama (INCAP). Incaparina is the name applied to vegetable protein mixtures, in which there is at least 25% protein of quality comparable to milk protein. In Guatemala, Mexico and Colombia, Incaparina formula 9B is in commercial production. This formula consists of 29% ground whole maize, 29% ground whole sorghum grain, 38% cottonseed flour, 3% torula yeast, 1% calcium carbonate, 4,500 I.U. vitamin A. per 100 grams, protein content 27.5%. Some choice in the main calorie components is allowed if local products are more available and cheaper.

The nutritive value of this mixture has been proved by amino acid analysis, many feeding trials in rats, chicks, and swine, and clinical tests in children, including treatment of kwashiorkor and nitrogen balance studies.

Incaparina can be drunk as an atole, which is made by adding water (one glass of water for 25 g of incaparina), some sugar, and cooking for 25 minutes. It can also be incorporated into bread, puddings, and soups. Incaparina is being sold in polyethylene bags containing 75 g at a cost of 3 U. S. cents per bag (Shaw, 1964).

In Brazil, with the assistance of UNICEF and technical advice from FAO and WHO, Fortifex, a 30% protein-rich food, is produced by the Nestle Company of Brazil. The formula consists of corn starch, soy flour, methionine at 0.2%, calcium barbonate, vitamin A, riboflavin, and thiamine. The results of rat and child feeding experiments demonstrated that Fortifex is a useful child feeding supplement and as a weaning food. The price is about 2 U. S. cents per 100 grams. Distribution is now being initiated on a large scale (Teply, 1964).

In Peru, the evaluation of fish protein concentrate, FPC, alone and in combination with wheat has been done by

Graham (Graham, Baertle, and Cordans, 1965). The combination of FPC with wheat is superior to FPC alone. Wheat noodles enriched with 10 percent fish protein concentrate were used as a dietary supplement in experiments and gave good results (Graham et al., 1966).

In Chile, there has been work at the University of Chile on an adaptation of fish flour for human nutrition and especially for infants. They have tested bread, some forms of pasta, and soup enriched with FPC and got encouraging results on the small groups tested (Möncksberg, 1966).

Europe

In Yugoslavia, a project to achieve the production of a low cost, cereal based, dry food mixture including vegetables for infants is underway with the assistance of UNICEF and technical advice from FAO and WHO (UNICEF, 1964).

U.S.A. and Canada

At Fayetteville, Sure (1957) added a small amount of defatted fish flour to milled wheat flour, corn meal, and rice, and found fish flour had high protein content and high biological value and improved the nutritive value of mixtures which can be used for infant feeding.

Sabby, Middleton, and Morrison (1962) found that the procedure used for defatting can markedly influence the nutritive value of fish flour to be used as a protein supplement to infant foods.

Do-Quang-Oanh (1963) proposed fortified foods for Vietnamese babies, in which rice at 70-80% of the diet was the basic ingredient. The protein content was brought up to 15% by the addition of soy, cod, egg, pork or a combination of soy and cod. The addition of sweet potato provided vitamin A, yeast provided vitamin B complex (except B_{12}), calcium carbonate and salt were added. From PER measurements, the cod and soy supplement appeared to be the best.

Summary

There are more plans, projects, and programs than action. Generally, large scale application of test results to improve the nutritional status of developing countries has not been accomplished. With two exceptions, all schemes have in common improved protein quantity and quality. Most schemes were designed primarily for pre-school children. Some used a mixture of vegetable and animal proteins, others used only vegetable proteins to increase the degree of utilization of proteins. All add one or more vitamins and minerals, and in one case a pure amino acid is added. Proteins for these schemes must come, in many cases, from supplies which are locally produced, but are already in short supply, other proteins must be imported (e.g. milk).

An exception is Incaparina which uses cottonseed protein, not formerly used for human consumption. All schemes have one or more (and usually more) ingredients which require sophisticated processing operations.

DESIGN OF THE EXPERIMENTAL DIETS

It seems clear that the nutritional problem of infants in the rice-eating countries of Asia (FAO, 1948; FAO, 1956; FAO, 1963) is many-fold. Improvements must be made in protein quantity and quality; vitamins must be added. In this study the main emphasis was placed on protein potency, the amino acid pattern of the diet. The solution was to try to find the best combination of vegetable and animal protein. Rice, the staple food in these areas, was used as the basic ingredient, the main calorie source. The protein level in the diets was raised to 15% as Davidson suggested (Davidson, Meiklejohn, and Passore, 1961) by the addition of soybean flour and cod fillet flour or egg powder. The nutritive values of these three animal proteins have been established (Patwardhon, 1961; Steinkraus, Van Buren, and Hand, 1961; Teply and György, 1962; Standal, 1963; DeMaeyer, 1965; Peeler *et al.*, 1951; Morrison, Sabby and Middleton, 1962; Pretorius and Wehmeyer, 1964; and WHO, 1965). Two per cent sweet potato was added as a source of provitamin A, 4% yeast as the source of vitamin B complex (Hock, Horn, and Dast, 1956; Scrimshaw and Behar, 1961); 0.5% calcium carbonate and 1.0% sodium chloride (see Table 2). The theoretical protein scores and nutritive values of the proposed diets were calculated from tables of McCance and Widdowson (1960), Orr and Watt (1957), and Watt and Merill (1963). Nutritive values of the proposed diets were about the same and met infant requirements, except for vitamin C (see Table 3).

TABLE 2.--Composition of diets, g/100 g.

Ingredient	Diet		
	COD-SOY	SOY-COD	SOY-EGG
Rice flour	83.7	74.8	73.0
Soybean flour	2.8	16.2	13.5
Whole egg powder			6.0
Cod fish flour	6.0	1.5	
Sweet potato	2.0	2.0	2.0
Yeast	4.0	4.0	4.0
$CaCO_3$	0.5	0.5	0.5
NaCl	1.0	1.0	1.0

Protein scores based on various standards (see Table 1) were plotted as a function of the amount of the main protein supplements as shown in Figure 1. The objective was to cover a range of combinations of an animal and vegetable protein with a limit on the total protein. Six percent and 1.5% cod fillet flour and 6% egg powder were selected as the amounts of animal protein for these

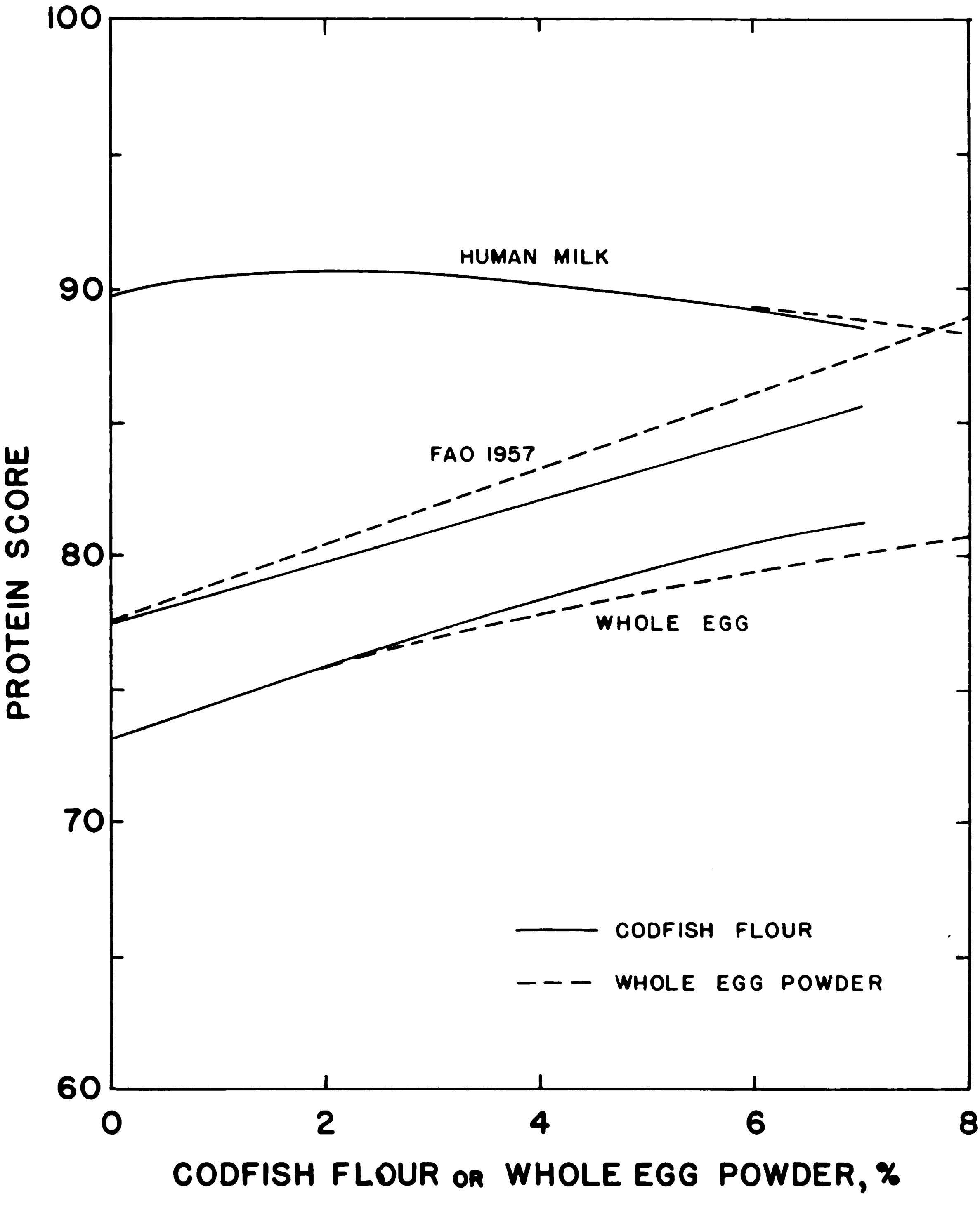

Fig. 1.--Protein score, based on three different standards (human milk, FAO 1957, and whole egg), as a function of the main protein supplement, total protein content at 15%. (Amino acid content from McCance and Widdowson, 1960, and Orr and Watt, 1957.)

TABLE 3.--Nutrients in the diets,[a] per 100 g.

Ingredient	Diet		
	COD-SOY	SOY-COD	SOY-EGG
Water, g	9.54	8.95	8.52
Protein, g	15.4	15.8	15.8
Fat, g	1.64	4.25	5.86
Ash, g	2.56	2.78	2.84
Crude fiber, g	0.75	0.99	0.93
Calcium, mg	240.0	260.0	270.0
Iron, mg	1.9	2.7	2.9
β-carotene, mg	0.97	0.99	1.04
Thiamine, mg	0.68	0.79	0.82
Riboflavin, mg	16.0	16.0	17.0
Niacin, mg	4.4	4.0	3.0

[a]Minerals and vitamins, except Vitamin A, were calculated from Watt and Merrill (1963). The rest of the nutrients were measured.

feeding trials. The rest of the protein was from soybean flour and, of course, rice, yeast, and sweet potato; total protein was 15%. In a sense, the ideal pattern was being tested at the same time, because, if human milk represented the ideal pattern, then there should be little or no difference between 1-1/2% and 6% cod; on the other hand, if FAO, 1957 or whole egg were the ideal, then 6% cod might be much better.

The proposed diets are intended to be cooked with water and are expected to have a flavor, texture, and consistency similar to rice gruel, the traditional infant food.

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EXPERIMENTAL

Preparation of Ingredients

Rice Flour

Commercial rice flour of unknown history was used.

Soybean Flour

Chippewa soybeans, 1964 crop, partially cleaned, were soaked in cold water overnight. About 10% of the skins of the beans were removed. Then the beans were washed and autoclaved in a steam retort at 110°F for 30 minutes. They were initially dried at 160°F for 5-1/2 hours in a Proctor and Schwartz cabinet drier and kept in the drier for three days. Following this treatment, on the fourth day, the beans were dried at 150°F for another two hours. During the drying period, the beans were turned over and the tray positions were changed in the drier once an hour to obtain a more uniform drying temperature. While still warm, the dried beans were ground in a Wiley mill, first through a No. 20 sieve and then through a No. 40 sieve. The soybean flour was prepared about 20 weeks before the first feeding test (Test 3) in the present study. The flour was stored in sealed glass bottles and refrigerated until used.

Codfish Flour

At 108 days before Test 3, frozen cod fillets were cooked in a steam kettle at boiling temperature for 20 minutes, including time to thaw, with occasional stirring. The cooked fish was drained by pressing lightly, through cheese cloth, and broken into coarse pieces by passing it through a food and meat chopper. Then the coarse pieces were placed on perforated trays, dried at 184-180°F for one hour and then at 158-162°F for two hours successively in the cabinet drier under cross air flow. The dried fish was ground in a Wiley mill first through a No. 20 sieve, then through a No. 40 sieve, and stored under refrigeration in sealed glass bottles.

Whole Egg Powder

A commercial product, glucose reduced and stablized, of unknown history was used.

Yeast

A commercial product, Anheuser-Busch chipped yeast, strain G was used.

Sweet Potato

The Goldrush, precooked dehydrated sweet potato flakes, provided by the Southern Regional Research Laboratory of the U. S. Department of Agriculture were used. In their procedure, the potatoes were preheated, lye peeled, and made into a puree of 20% solids with water, to which

0.01% sodium sulfate, 0.04% sodium bisulfate, and 0.1% antioxidant Tenox VI (all dry basis) were added. The puree was dried on a drum drier for 17 seconds. The dehydrated sweet potato flakes were packed in tin cans and sealed in an atmosphere of nitrogen (Deobald et al., 1962). Before use in these studies the flakes were ground in a Wiley mill through a 40 mesh sieve.

Dog Chow

A commercial food, Purina Dog Chow, ground in a food and meat chopper, was used.

Calcium Carbonate

A chemically pure grade was used.

Sodium Chloride

Diamond crystal salt, food grade, was used.

CASEIN Diet

A standard CASEIN diet suggested by Campbell (1963) was used in the animal feeding trials as a means of comparing PER data from different laboratories and of avoiding or correcting for variations in technique from one experiment to another. The 10% protein content special CASEIN diet was prepared by General Biochemicals, Chagrin Falls, Ohio. The diet consists of 67.7% corn starch, 12.3% casein, 10.0% corn oil, 5.74% non-nutritive fiver, 4.00% sale mix U.S.P. XIV, and 0.26% vitamin mix.

Salt mix U.S.P. XIV consists of 0.009% ammonium alum, 11.280% calcium biphosphate, 6.860% calcium carbonate, 30.830% calcium citrate, 0.008% cupric sulfate, 1.526% ferric ammonium citrate, 3.520% magnesium sulfate, 0.020% manganese sulfate, 12.470% potassium chloride, 0.004% potassium iodide, 21.880% dibasic potassium phosphate, 7.710% sodium chloride, and 0.050% sodium fluoride. The vitamin content in 100 pounds of CASEIN diet is 2.2700 g vitamin A, 0.1135 g vitamin D, 4.1270 g DL-alpha tocopherol, 0.2270 g menadione, 0.2270 thiamine hydrochloride, 0.4540 g riboflavin, 0.1816 g pyridoxine hydrochloride, 1.8160 g calcium pantothenate, 1.8160 g niacin, 90.8000 g choline chloride, 11.3500 g inositol, 4.5400 g para-amino benzoic acid, 0.9080 g vitamin B_{12} (0.1% trituration with mannitol), 0.0091 g biotin, and 0.0908 g folic acid. The proximate analysis of the CASEIN diet was 7.45% moisture, 2.71% ash, 5.69% crude fiber, 10.35% protein, 11.84% fat.

All ingredients, Dog Chow, CASEIN diet and the proposed diets were stored in a 3-5°C cool room before chemical analysis and feeding trials.

Chemical Analysis of Ingredients

The nutritive values of the proposed diets are dependent upon their ingredients. A proximate analysis of materials was carried out by the methods described below;

the results are summarized in Table 4 (all analyses except β-carotene followed AOAC, 1960, methods or modifications thereof).

Moisture

Duplicate 10-g, well mixed samples of all ingredients were accurately weighed in cool moisture dishes, dried in an electric oven at 105 ± 1°C for 5 hours, transferred to a desiccator, and weighed after half or one hour when they had cooled to room temperature. The residues are reported as total solids and loss in weight as moisture. The residues were used for ether extraction.

Ash

Duplicate 5-g samples were accurately weighed into ash crucibles, carbonized first under a Bunsen burner, then put into a muffle furnace which was preheated to 600°C, and held at this temperature for two hours. The crucibles were transferred directly into a desiccator, cooled to room temperature, and weighed immediately. The remaining weight is referred to as ash.

Crude Fat

Duplicate 10-g samples, residues from the moisture determination, were extracted with petroleum ether in a Soxhlet apparatus for 16 hours. The petroleum ether was evaporated under vacuum. The flasks were kept in a desiccator overnight and then weighed. The percentage of fat is referred to the original sample weight.

TABLE 4.--Proximate composition of the ingredients, g/100 g.

Ingredient	Water	Protein	Fat	Ash	Crude Fiber
Rice flour	11.0	7.52	0.99	0.81	0.63
Soybean flour	4.92	35.6	18.3	4.24	1.90
Cod fish flour	3.24	91.1	1.08	4.35	0.00
Whole egg powder	4.28	46.8	41.9	3.56	0.00
Sweet potato	3.84	8.17	1.00	4.13	4.40
Yeast	5.11	52.1	1.92	6.45	3.28

Protein

Duplicate 0.7-2.2 g samples were accurately weighed and put into digestion flasks to which 0.7 g HgO, 15 g anhydrous Na_2SO_4 and 25 ml H_2SO_4 were added. Two or three "tamer tabs" were added for taming the boiling action. Flasks were placed in an inclined position and heated until frothing ceased. Samples were hydrolyzed at least 30 minutes beyond the time at which the solutions became clear. The solutions were cooled below 25°C, 200ml cool water and 25 ml thiosulfate solution were added, and mixed to precipitate the mercury. A few zinc granules were added to prevent lumping. The flasks were tilted and 25 g solid NaOH were added without agitation. The flasks were immediately connected to the distilling bulbs on the condensers and the tips of the condensers were immersed in standard acid solutions in the receivers. Then the flasks were rotated to mix the contents thoroughly, and heated until all NH_3 had distilled (at least 150 ml distillate). The excess standard acid in the distillate was titrated with NaOH, using methyl red as an indicator. The results were corrected with a blank. Factors to convert g N to g protein were taken from Orr and Watt (1957).

Crude Fiber

Duplicate 2-g samples, residues from ether extraction, were used. To the samples in the digestion flasks were added 0.5 g asbestos, and 3 drops dilute Dow Corning Antifoam A emulsion (1 + 3) and 200 ml of boiling 1.25% H_2SO_4 solution. The flasks were immediately connected with the reflux condensers, rotated frequently until the samples were thoroughly wetted, and heated exactly 30 minutes. Samples were kept in contact with the solution. After 30 minutes, the flasks were promptly removed and the solutions were filtered through linen in fluted funnels; the samples were washed with boiling water until the washings were no longer acid. Then the samples were returned to the flasks to which 200 ml boiling 1.25% NaOH solution were added. The flasks were connected with the reflux condensers again and boiled exactly 30 minutes. Then the flasks were removed immediately, and the solutions filtered through Gooch crucibles which were prepared with asbestos mats. The residues were washed with hot 10% K_2SO_4 solution whenever the filtration became difficult. After being thoroughly washed with boiling water and 15 ml alcohol, the crucibles and contents were dried at 105°C for 5 hours to constant weight, then cooled in a desiccator and weighed. The contents of the crucibles were ignited in an electrical muffle furnace at 600°C, then cooled in a desiccator and weighed. The loss in weight was reported as crude fiber.

Carbohydrate

Carbohydrate was not determined, but the calorie content of the diets was estimated. The carbohydrate was assumed to be the difference between total weight and the sum of water, protein, fat, ash, and crude fiber. The calorie calculation was based on the Atwater system using energy values from Watt and Merrill (1963). The results were COD-SOY 360, SOY-COD 366, SOY-EGG 377, and CASEIN 401 cal/100 g.

β-carotene (Wail and Kelly, 1943)

Duplicate samples (10 g in the case of soybean flour, 1 g in the case of sweet potato flakes) were accurately weighed in 100 ml beakers. Thirty milliliters of hot distilled water were added and mixed well with the samples. After 20-30 minutes hydration, 75 ml 95% ethanol were added to the samples and then stirred thoroughly and left overnight in a dark room. Thirty milliliters Skellysolve B (normal hexane) was added to the beakers and stirred thoroughly. The solutions were transferred and passed through glass fritted filters, which were connected to suction flasks. The residues were washed with alternate portions of 30 ml alcoholand 30 ml Skellysolve B until colorless filtrates were obtained. The filtrates were transferred into 500 ml separatory funnels to which 100 ml H_2O and 50 ml 5% Na_2SO_4 were added. The funnels were swirled and turned upside down while the stoppers were held and the stopcocks were opened to release pressure. The lower aqueous alcoholic solution was extracted three times with 30 ml Skellysolve B and 100 ml water. The Skellysolve layer was filtered through a filter paper containing 1 teaspoonful of anhydrous sodium sulfate into an Erlenmeyer flask containing a small amount of anhydrous sodium sulfate. Then the contents were condensed to 25 ml on a steam bath; the filtrate was transferred into an absorption column prepared with a mixture of three parts Hyflo Supercel and one part activated magnesium. After the sample had all been transferred to the column, the carotene was eluted by pulling a Skellysolve B-acetone mixture (95:5) through the column. About 50-70 ml of solvent was required after the first color came through. The elute was brought up to 100 ml with Skellysolve B and the transmittance was measured with an Evelyn colorimeter at 440 mμ. The β-carotene content was determined from a standard curve.

For dry egg powder, duplicate 1 g samples were weighed in 150 ml beakers to which 4 ml H_2O was added. The samples were stirred to a paste, and 50 ml acetone was added, a little at a time, starting with about 5 ml. After standing for 5 to 10 minutes, samples were filtered through Watman No. 4 filter paper and washed thoroughly with acetone. Filtrates were collected in 100 ml volumetric flasks and brought up to 100 ml with Skellysolve B. The transmittance was measured in an Evelyn colorimeter.

Ingredients had been stored in a refrigerator for two months before analysis. The β-carotene in the dehydrated sweet potato flakes was 50.5 mg/100 g, egg powder 2.15 mg/100 g, soybean flour 0.137 mg/100 g.

Bioassay

The proposed diets were evaluated before and after storage by bioassay in Test 3[1] and Test 5. Since 15% protein level of a high quality protein of proposed diets

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[1]The first two feeding experiments in this project of which the present study is a part were presented by Do-Quang-Oanh (1963).

were evaluated at 10% protein in Test 4. The protein level was reduced to 10% by adding corn starch to the diets.

In all feeding trials, male weanling rats of Holtzman strain were used. First the diets were assigned randomly to the cages and some adjustments were made to avoid what seemed to be too many cages on a side or a tier for one diet. Each rat as it was removed from the shipping box was weighed and assigned to a cage at random. Some adjustments were made upon receipt and just before the rats were put on the test diet to get equal average weights among groups. The diets were prepared from ingredients by mixing them in a Patterson-Kelley Liquid-Solids Twin Shell Blender for 15 minutes. In all three testing periods, rats were kept in the same room in which the lights were turned on from 8:00 A.M. to 5:00 P.M. every day during the trial. All rats were kept in individual cages (9.5" x 8" x 7.5") with screen bottoms, and received food in Franke jars (capacity about 65 ml) and water *ad libitum*. Food consumption was recorded every other day; the amount of spilled food was estimated. In the large majority of cases, 1/2 g or less was spilled in any 2-day period. A few rats spilled as much as 6 g/day; when a rat had this habit, his spilled food was collected and weighed. CASEIN diet was subject to more spilling than any of the others. Each rat was weighed to the nearest half gram every four days.

They received fresh water every other day. In Tests 3 and 5, respiratory infection was widespread, but much less so in Test 4; however, there seemed to be no complete correlation between weight gain and infection. The rats were killed at the end of the test periods.

Third Feeding Experiment

Rats 23 days old were kept in a room maintained at 76-80°F. Upon receipt, the rats were fed Dog Chow for one day. There were 12 rats on each test diet and the control group which was fed with the standard CASEIN diet. The initial weights of the rats at zero days (September 23, 1965), average 57.7 g, range from 54-63 g, were not significantly different among groups. The growth response of the rats was tested for 28 days and is plotted in Figure 2. Performance is summarized in Table 5 from reduced data, reduced by discarding the results from five very sick rats. There was no difference (5% level) in PER among COD-SOY, SOY-COD and SOY-EGG (EMS = 0.026). All of these three diets were good as judged by PER and growth rate.

Fifth (storage) Feeding Experiment

The same diets used in the third test, COD-SOY, SOY-COD, SOY-EGG, and CASEIN, were stored at high humidity (100% relative humidity) and high temperature (35°C) for 145 days. The diets, already mixed, were sealed in large

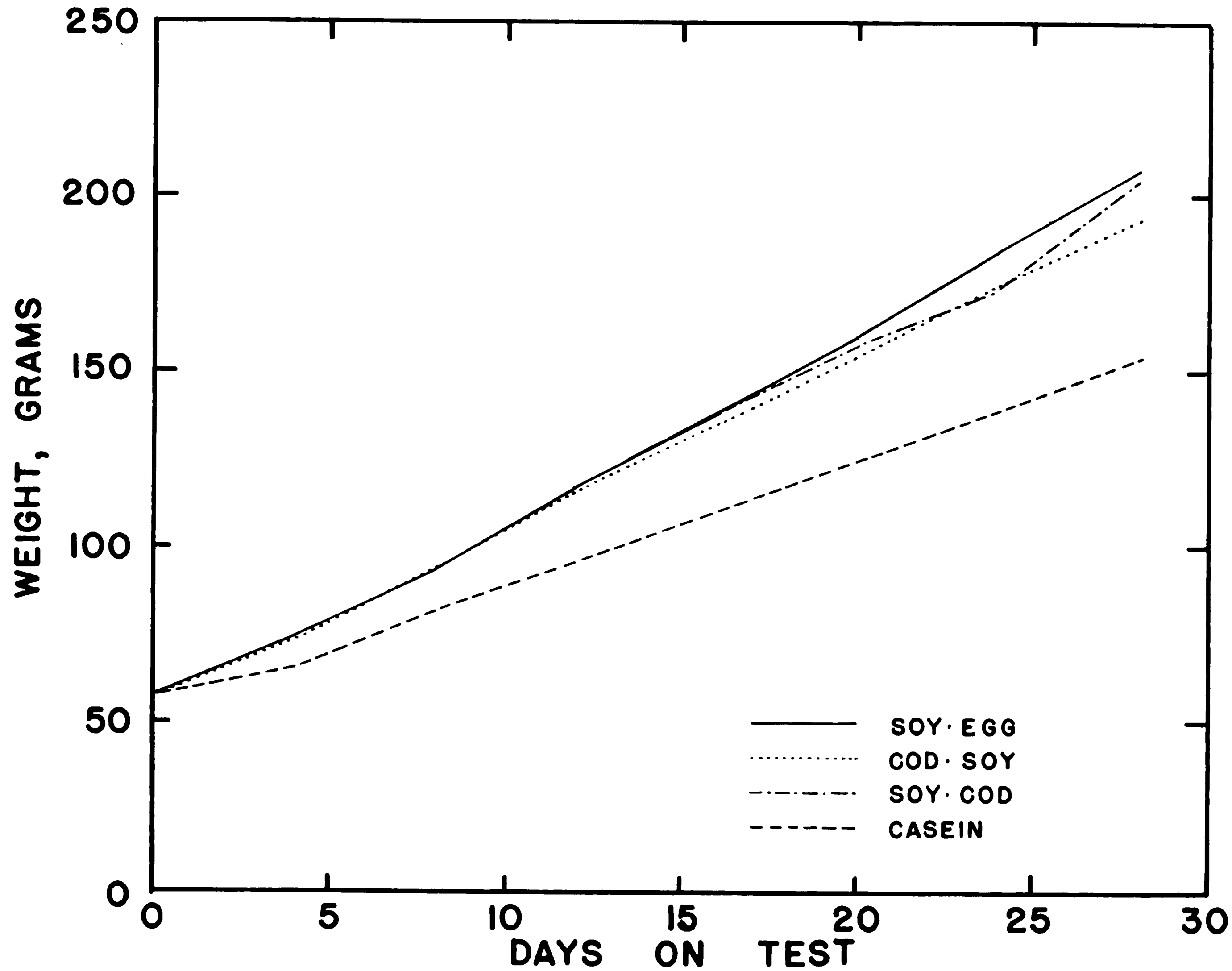

Fig. 2.--Growth curves of male weanling rats on fresh diets (Test 3--15% total protein, except CASEIN at 10%).

TABLE 5.--Test 3--Performance of male weanling rats on fresh test diets. (Total protein 15%, except CASEIN, 10%; 28 day test period.)

Diet	No. of rats	Weight gain g/day	Food Consumption g/day	Food Efficiency g gained/ g consumed	Food Efficiency g gained[a]/ kcal	PER g gained/ g protein
CASEIN	10	3.4	12	0.28	0.70	2.8
COD-SOY	9	4.9	14	0.34	0.94	2.2
SOY-COD	12	5.3	14	0.37	1.0	2.3
SOY-EGG	12	5.3	14	0.38	1.0	2.4

[a]Calculation of energy value by the Atwater system, using energy values from Watt and Merrill (1963) and carbohydrate content calculated by difference from the proximate analysis of ingredients.

cans with an amount of water in an open beaker, calculated to give a final moisture content of 17%. After removal of the mold on the upper layer, the stored diets were mixed in the Twin Shell Blender for 15 minutes and fed to the rats. The two control groups were fed fresh CASEIN and Purina Dog Chow. Rats 23 days old were divided into six groups at random, nine rats on the stored diets and fresh CASEIN, three rats on Dog Chow; temperature was 70-72°C. Prior to the test, the rats were fed Dog Chow for 20 hours, then put on the test diets. The average initial weights at zero days, March 18, 1966, 59 g, range from 53.5 to 65.5 g, were not significantly different among groups. The test period was 28 days. The growth curves of rats on the test diets are shown in Figure 3. The performance is summarized in Table 6; no results were discarded. There was no significant difference (5%) among CASEIN-STG, COD-SOY-STG, SOY-COD-STG, and SOY-EGG-STG, but CASEIN-STG was significantly lower than fresh CASEIN.

Fourth Feeding Experiment

The proposed diets were reduced to 10% protein by adding corn starch and 1/3 the amount of vitamin and mineral mixture used in the CASEIN diets. One new diet, SOY-10, using soybean flour as the only protein source, except protein from yeast, rice and sweet potato, was introduced into the experiment. One control group was fed the standard CASEIN diet, another group was fed Dog Chow.

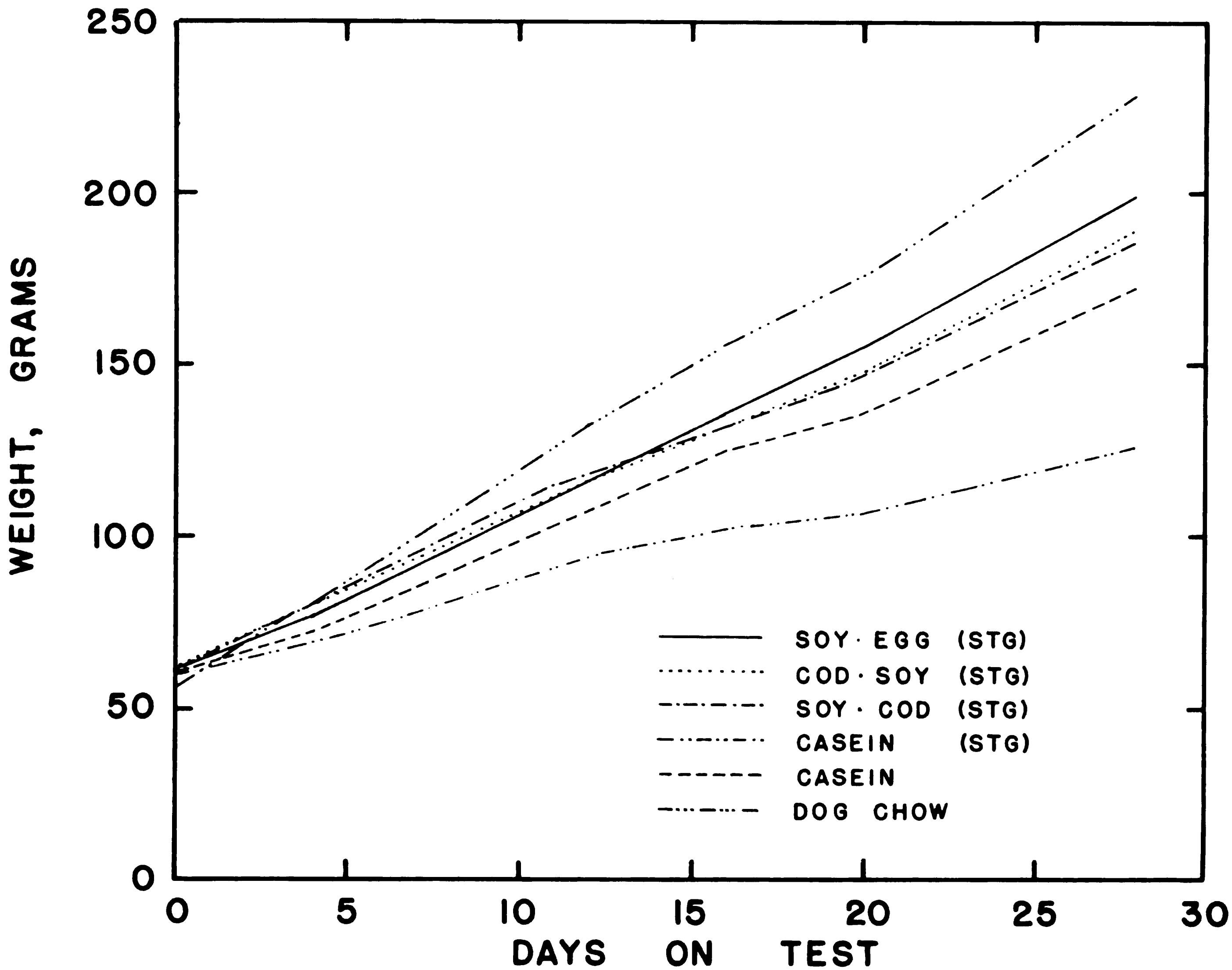

Fig. 3.--Growth curves of male weanling rats on stored diets (Test 5--stored for 145 days at 100% R.H. and 95°F; 15% total protein, except CASEIN at 10% and Dog Chow at 24.5%).

TABLE 6.--Test 5--Performance of male weanling rats on stored test diets. (Total protein 15%, except CASEIN diets 10%, and Dog Chow 24.5%; 28 day test period).

Diet	No. of rats	Weight gain g/day	Food Consumption g/day	Food Efficiency		PER
				g gain/ g consumed	g gained[a]/ kcal	g gained/ g protein
CASEIN (fresh)	9	4.0	14	0.27	0.70	2.7
CASEIN-STG	9	2.4	11	0.22	0.58	2.2
COD-SOY-STG	9	4.6	15	0.31	0.92	2.1
SOY-COD-STG	9	4.5	15	0.31	0.92	2.2
SOY-EGG-STG	9	5.0	15	0.34	0.95	2.3
DOG CHOW(fresh)	3	6.1	17	0.36	1.1	1.5

[a]Calculation of energy value by the Atwater system, using energy values from Watt and Merrill (1963) and carbohydrate content calculated by difference from proximate analysis of ingredients.

Rats 22 days old were divided into six groups at random, eight rats each on test diets, three rats for Dog Chow, and kept in a room maintained at 72-73°C. The initial weights at zero days, November 23, 1965, average 59.4 g, range 55-68 g, were not significantly different among groups. The test period was 26 days. The growth response of rats on the test diets and Dog Chow are shown in Figure 4. The performance is summarized in Table 7; results from one sick rat were discarded. The COD-SOY-10 and SOY-EGG-10 were superior to SOY-COD-10 and SOY-10 (EMS = 0.065).

Storage and Palatability

The three proposed diets and standard CASEIN diet were stored at 100% RH, 35°C for 145 days. Ideally, the diets should be raised at once to a high moisture, perhaps 15-16%, just before they are placed in the high humidity and high temperature envionrment. Since there seemed to be no simple method of adding moisture which would not require additional processing (redrying, for instance), the method used was to expose the product to a free water surface in a closed container. Though not ideal, this exposure and gradual increase in moisture content is very like the situation one might expect in a poorly packaged product sitting on a shelf in a damp environment. After 145 days nearly all the water had evaporated from the beakers. In COD-SOY from an original 350 g of water, all

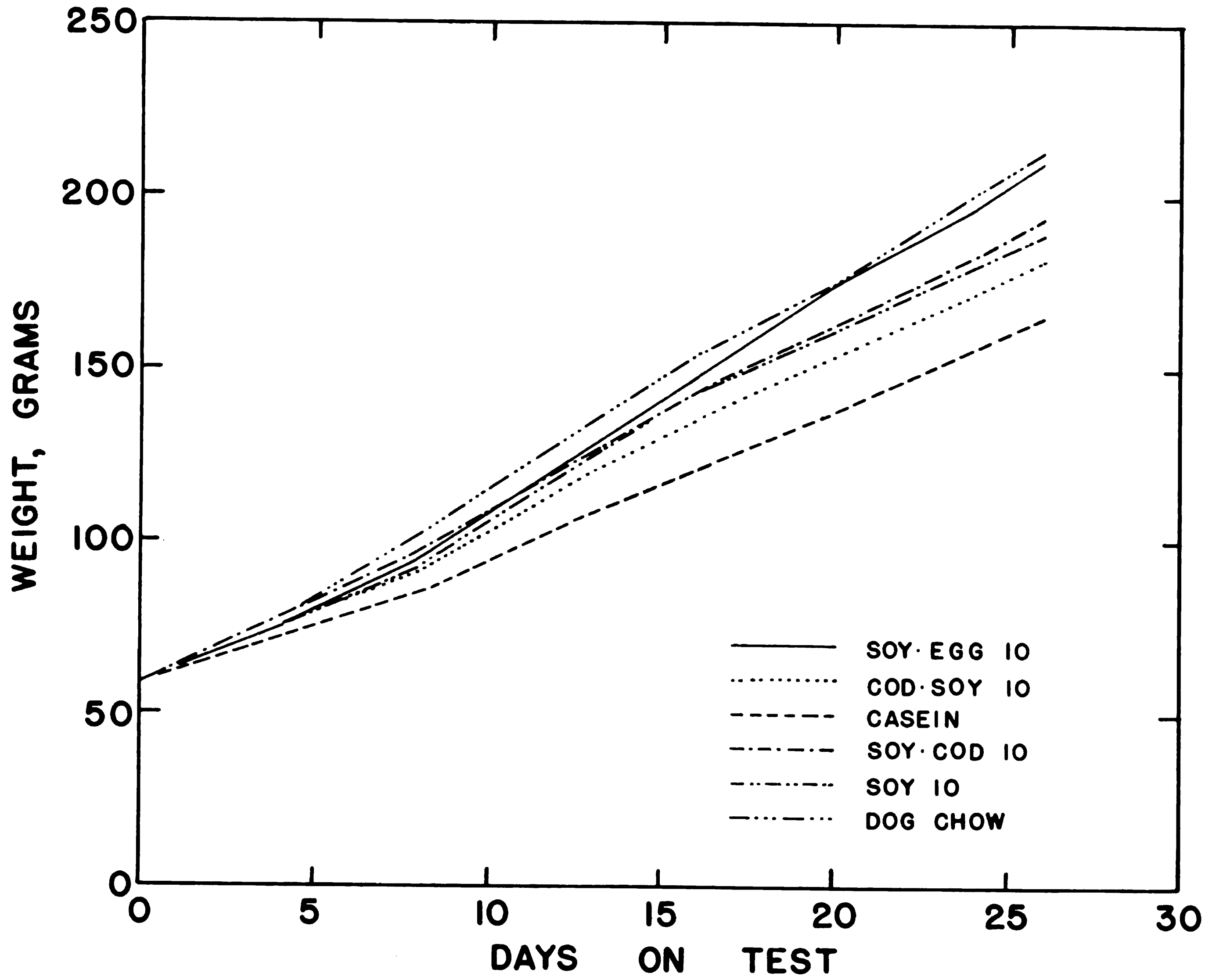

Fig. 4.--Growth curves of male weanling rats on fresh diets at 10% total protein, except Dog Chow at 24.5% (Test 4).

TABLE 7.--Test 4--Performance of male weanling rats on fresh test diets. (Total protein 10%, except Dog Chow, 24.5%; 26 day test period.)

Diet	No. of rats	Weight gain g/day	Food Consumption g/day	Food Efficiency		PER, g gained/ g protein
				g gained/ g consumed	g gained[a]/ kcal	
CASEIN	8	4.1	14	0.29	0.71	2.9
COD-SOY-10	7	5.1	16	0.31	0.85	3.1
SOY-COD-10	8	5.2	18	0.29	0.84	2.9
SOY-EGG-10	8	5.8	18	0.33	0.86	3.3
SOY-10	8	5.0	18	0.28	0.75	2.8
DOG CHOW	3	5.9	16	0.38	1.3	1.5

[a]Calculation of energy value by the Atwater system, using energy values from Watt and Merrill (1963) and carbohydrate content calculated by difference from proximate analysis of ingredients.

had evaporated; in SOY-EGG of 422 g of water, all but 17 g had evaporated. As expected, the moisture content varied from top-to-bottom in the containers, in SOY-EGG 17.1% at the top and 14.1% at the bottom. The surfaces of COD-SOY and SOY-EGG were quite moldy, but the SOY-COD was less so. This moldy, caked layer was removed before the feeding tests. There seems no doubt that some of the off-flavor and odor (discussed below) was due to the mold. The measured final moisture (Table 8) was the same or greater than that calculated from water absorbed from the beakers, so leakage from the cans is assumed to have been negligible.

The physical and chemical properties were determined before and after storage. Moisture, protein, fat, rancidity, color, carotene, and palatability of the diets were tested in the dry or cooked form, as appropriate. The results, except palatability, are summarized in Table 8. The methods of protein, fat, and carotene analysis for the diets were the same as for ingredients. The other methods used are given below.

TBA Test

A distillation method was used (Tarladgis et al., 1960). Duplicate 10-g samples were transferred quantitatively into 500 ml Kjeldahl flasks with 97.5 ml of distilled water. Two and one-half milliliters of 4N HCl and a small amount of Dow Antifoam A were added to the

TABLE 8.--Physical and chemical determinations of proposed and standard diets before and after storage (100% R.H., 95°F).

Diet	COD-SOY		SOY-COD		SOY-EGG		CASEIN	
Storage time, days. . .	0	145	0	145	0	145	0	145
Property:								
Moisture, % w.b.	9.5	15	9.0	16	8.5	14	7.4	13
TBA absorbance at 540 mμ	0.0	0.15	0.0	0.04	0.0	0.03	0.0	0.10
Peroxide no. meq/1000 g oil	0.0	0.0	0.0	0.0	10	10	40	230
β-carotene, mg/100 g	0.96	0.40	0.99	0.52	1.04	0.74	--	--
Color[a]								
Dry Food								
L . . .	84.4	76.2	83.1	73.1	82.3	73.0	87.8	82.1
a_L. . .	2.2	1.5	1.9	2.6	2.1	3.1	0.1	0.2
b_L. . .	12.9	12.0	14.2	14.3	17.6	16.2	9.3	10.5
Cooked Food								
L . . .	63.1	55.1	63.4	56.2	64.0	57.5	62.5	62.6
a_L. . .	- 4.5	- 2.5	- 3.5	- 0.8	- 3.9	0.9	- 7.1	- 5.7
b_L. . .	12.8	12.7	14.4	14.2	16.5	17.3	3.6	6.6
Absorbance at 460 mμ	37	31	46	35	69	59	--	--

[a]Gardner color difference meter, referred to white: L = 90.9; a_L = -2.0; b_L = +2.5.

flasks. The distillation was done at the highest temperature setting for about 10 minutes, and 50 ml distillate was collected. Five milliliters of distillate were mixed with 5 ml of 0.02M TBA solution (dissolved in 90% glacial acetic acid) in a glass stoppered tube. The tube was immersed in a boiling water bath for 35 minutes and then cooled. The transmittance or optical density was measured at 540 mμ with a Spectronic 20 spectrophotometer against a blank of distilled water and TBA solution.

Peroxide Number (Wheeler, 1932)

Duplicate 5-g samples of the extracted oil from stored diets were transferred to 250 ml Erlenmeyer flasks and dissolved in 30 ml of solvent mixture (glacial acetic acid : chloroform = 3 ; 2 by volume) and one ml of saturated KI was added. The flasks were shaken in a rotary motion for exactly two minutes, and 50 ml water was added. Then the liberated iodine was titrated with 0.1 N thiosulfate by using 0.5 ml starch solution as an indicator. The peroxide number was expressed in terms of milliequivalents per 1000 grams of oil.

Color Determination

The valuation of color was by transmittance and reflectance measurements. Samples for transmittance measurement were prepared by soaking 5 g of sample in 10 ml c.p. acetone for exactly five minutes with occasional stirring. The residues were filtered through Whatman No. 2 filter paper and washed three times with 2 ml portions of acetone. The transmittance was measured on a Bausch and Lomb Spectronic 20 colorimeter from 400 mμ to 540 mμ. There was no sharp absorption peak in this range, but there was an ill-defined maximum near 460, before storage, which shifted to about 445 after storage.

The reflectance was measured with a Gardner Color Difference Meter for both dry and cooked food. The machine was standardized against a white standard plate ($L = 90.9$; $a_L = -2.0$; $b_L = +2.5$). Dry foods were measured directly by covering the bottom of a petri dish with sample to a depth of about 1/4". The cooked foods were prepared by the following method. Duplicate 10-g samples were mixed with 100 ml distilled water in a 250 ml beaker and cooked on a Chromalox hot plate. Total cooking time was 12 minutes. The samples were stirred constantly with glass stirring rods. Then the cooked foods were transferred, for color measurements, to a glass petri dish and filled to a depth of about 1/4 inch.

Taste

The effect of rancidity on acceptance cannot be detected by any chemical method, so taste must be relied on. Foods were prepared by cooking with tap water for 12 minutes (water : food = 10 : 1) on a Chromalox hot plate, the same method described above for color measurements. Eight samples, two of each diet COD-SOY, SOY-COD, SOY-EGG, and CASEIN, one of the "fresh" material (stored under refrigeration) and one of material stored at high humidity and high temperature, were cooked. The cooked, stored diets were terrible, except SOY-COD-STG, which was not much different in color, taste, odor, or texture from fresh SOY-COD. It was judged edible, but it did have a slight "old" odor. COD-SOY-STG had a musty smell, like an old barn, and the taste was very bitter, sufficient to make it inedible. The odor of SOY-EGG-STG was somewhat musty, better than COD-SOY-STG, but unsatisfactory. CASEIN-STG was bitter, darker than the fresh CASEIN and had a tallowy taste.

Eight people were asked to make informal odor evaluations of the uncooked fresh and stored diets, and to make a choice among fresh diets and among the stored diets.

The stored diets were called stale, moldy, rancid, sour or old rice bran smell; the fresh samples were either preferred or called indistinguishable from the stored. Some thought the fresh COD-SOY too "fishy." Four judges preferred SOY-EGG to COD-SOY and SOY-COD, the other four expressed no preference at all, but found all three diets acceptable, in general. All agreed that the diets were similar to rice flour in appearance.

RESULTS AND DISCUSSION

The diet designs were dependent upon a theoretical calculation of protein score, an index of protein quality evaluation. But protein score changes according to the standards, and, as of today, the ideal pattern, specific for infants, is not available. In a sense, the experimental design, based on suggested amino acid patterns for infants is a test of the suggested patterns themselves. But any conclusion about the standards is limited by the fact that weanling rats, used in the feeding trails, can not exactly represent infant requirements. It might be possible to work out the ideal pattern by computer, and protein score might eventually prove to be a simple and reliable approach for protein evaluation.

In Test 3, a bioassay was used to test the proposed diets and a special CASEIN diet at 10% protein level. This CASEIN diet, suggested by Campbell (1963), was used to measure variation in the experimental conditions from one experiment to another. All proposed diets were designed and fed at 15% total protein because if 15% of the energy requirement is supplied by a high quality protein, the infant protein requirement should be met. There was no statistical difference at 5% among the three proposed diets (Table 5) probably because, at 15%, the high quality proteins of these diets are not at a critical level for rats. Figure 2 shows that all diets are good for growing rats but inferior to CASEIN in terms of PER. Moreover, according to the food efficiency values, SOY-COD and SOY-EGG are superior to COD-SOY. Although all diets were expected to be good, the SOY-EGG should be the best or equal to COD-SOY according to protein score (see Figure 1) but, for reasons mentioned, no proof was available from this test.

The diets, after storage at high temperature and humidity for 145 days, still gave good growth to rats (see Figure 3). Off-flavor did not affect consumption. Consumption was higher but, on a solids basis, was somewhat lower, and food efficiency decreased (see Table 9). The PER values of these diets were not significantly affected, probably because of the high protein content. Note that the CASEIN-STG died did show a significantly reduced PER. According to informal taste studies, all proposed diets except SOY-COD are objectionable. SOY-COD is just acceptable probably due to the better keeping quality of plant protein.

All diets after storage were darker, probably due to browning reaction (see Table 8); on the other hand, color change by transmittance showed bleaching, probably due to breakdown of β-carotene.

TABLE 9.--Summary of performance of male weanling rats on test diets before and after storage (100% R.H., 95°F; total protein 15%, except CASEIN 10%; 28 day feeding period).

	Food Consumption g/day		Food Efficiency g gained/ g consumed		Food Efficiency g gained/ kcal		PER, g gained/ g protein	
Storage time, days. .	0	145	0	145	0	145	0	145
Diet:								
COD-SOY	14	15	0.34	0.31	0.94	0.92	2.2	2.1
SOY-COD	14	15	0.37	0.31	1.0	0.92	2.3	2.2
SOY-EGG	15	15	0.38	0.34	1.0	0.95	2.4	2.3
CASEIN	12[a]	11	0.28	0.22	0.70	0.58	2.8	2.2
	14[b]		0.27		0.70		2.7	

[a]Test 3. [b]Test 5.

Test 4 was carried out by reducing the protein content in the diets to 10% with corn starch and by adding a small amount of the same vitamin and mineral mixture used in the standard CASEIN diet, because, with high quality proteins, rats are insufficiently stressed when both quality and quantity are high. Another diet, SOY-10, having all supplements except cod fish flour or whole egg powder, was introduced to test whether vegetable protein alone is inferior to the mixtures of vegetable and animal protein. The results are given in Table 7 and Figure 4. All proposed diets were equal or superior to CASEIN in PER, weight gain, and food efficiency, and they provided good growth to male weanling rats. Among these diets, SOY-EGG-10 and COD-SOY-10 were superior to SOY-COD-10 and SOY-10, probably due to better amino acid content. This result is consistent with the protein scores based on FAO 1957 and whole egg patterns (see Figure 1). With sufficient vitamin and mineral supplements, the SOY-10 can be nearly as good as the other mixtures. In terms of food efficiency, all proposed diets at 10% protein level are less efficient than those at 15%.

For a more comprehensive evaluation of the proposed diets, a series of experiments would be of interest, for example, diets, all with the yeast-sweet potato-salt supplements, all at 10% total protein, and ranging in the ratio of cod fish flour to soybean flour from all codfish to all soybean. A protein free diet, as suggested by Hegsted and Chang (1965) could be used to determine the relative growth index as an evaluation of protein quality of the diets.

SUMMARY AND CONCLUSIONS

A summary of the performance of male weanling rats on diets containing soybean flour is given in Table 10. At 15% protein level, the proposed diets, the animal protein or mixture of vegetable and animal protein, gave better results than SOY-ONLY. The protein quality of soybean can be improved by adding a small amount of animal protein, codfish flour, or egg powder. At 10% protein level, SOY-EGG-10 and COD-SOY-10 are better than SOY-COD-10 or SOY-10 probably due to amino acid content, a deciding factor.

Table 11 is a summary of the performance of male weanling rats on diets containing codfish flour. There is no statistical difference between SOY-COD and COD-SOY or between PAP and COD at 15% total protein in terms of PER values. Therefore, a proper combination of vegetable and animal protein can give the same benefit as animal protein only. The proportion of the animal protein in PAP is just over half that in COD; the proportion in SOY-COD is less than 1/3 that in COD-SOY.

The proposed diets were dry flour-like products, similar to rice flour in appearance. They are prepared for eating by cooking with water into a porridge. A 9.1

TABLE 10.--Summary of performance of male weanling rats on diets containing soybean flour.

Diets	Test	No. of rats	% Total protein from soy flour	Food Efficiency		PER, g gained/ g protein
				g gained/ g consumed	g gained/ kcal	
15% total protein						
COD-SOY	3	9	6.7	0.36	1.0	2.4
SOY-EGG	3	12	32	0.38	1.0	2.5
SOY-COD	3	12	38	0.37	1.0	2.4
PAP	2	6	47	0.40	1.1	2.7
SOY	1	6	50	0.26	0.70	1.7
	2	4	50	0.30	0.81	2.0
SOY-ONLY	1	6	63	0.19	0.50	1.2
10% total protein						
COD-SOY-10	4	7	6	0.31	0.85	3.1
SOY-COD-10	4	8	38	0.29	0.84	2.9
SOY-EGG-10	4	8	32	0.33	0.86	3.3
SOY-10	4	8	50.	0.28	0.76	2.8

TABLE 11.--Summary of performance of male weanling rats on diets containing codfish flour.

Diets	Test	No. of rats	% Total protein from cod flour	Food Efficiency		PER, g gained/ g protein
				g gained/ g consumed	g gained/ kcal	
15% total protein						
SOY-COD	3	12	10	0.37	1.0	2.4
PAP	2	6	24	0.40	1.1	2.7
COD-SOY	3	9	36	0.36	1.0	2.4
COD	1	6	41	0.38	1.0	2.6
	2	2	41	0.40	1.1	2.7
10% total protein						
SOY-COD-10	4	7	10	0.29	0.85	2.9
COD-SOY-10	4	8	36	0.31	0.84	3.1

kg infant (about one year old) has to take 250-300 grams of the diet per day to meet his nutritional requirements, except vitamin D and vitamin C. Citrus juice or green leafy vegetable juice is suggested as an additional supplement to be fed to babies to provide enough vitamin C. By proper exposure to sunshine the child would be able to get enough vitamin D.

The cost of the proposed diets will necessarily be more expensive than rice flour. But 73-84% of the diet is from rice, and the other ingredients, sweet potato, soybean, fish, yeast, are already available or are products native to Asia. In practice, to reduce costs and eliminate objections to fishy taste and odor, FPC, deodorized and defatted, should replace the codfish flour used in these tests.

The comparison of these proposed diets with the results of others is given in Table 12. According to food efficiency, all proposed diets are better than Indian Multipurpose Food, but not as good as Incaparina 8 or modified 8. All proposed diets give better PER values than that of rice, wheat, maize, bengal grain, soybean, and even skim milk, but not as good as that of CASEIN. The proposed diets of the present study are good enough for infants and should be acceptable, easily transported, kept and stored (moisture content 8.5%-10.0%) provided

TABLE 12.--Performance of weanling rats on various diets.

Diet	Protein Content	PER, g gained/ g protein	Food Efficiency, g gained/ g food	Reference
Rice	5	1.7	0.085	Patwardham, 1961
Wheat	5	1.3	0.065	Patwardham, 1961
Maize	5	1.0	0.050	Patwardham, 1961
Bengal gram	10	1.1	0.110	Patwardham, 1961
Black gram	10	1.0	0.100	Patwardham, 1961
Soybean	10	1.21±0.10	0.121	Steinkrans _et al._, 1961
Soybean	20	1.39±0.045	0.378	Steinkrans _et al._, 1961
Soybean	10	0.9	0.090	Patwardham, 1961
Skim milk	10.6	2.13	0.226	Subrahmanyan _et al._,1961
Skim milk	20	1.48±0.03	0.296	Steinkraus _et al._, 1961
Skim milk	10	1.59±0.05	0.159	Steinkraus _et al._, 1961
Casein	10.9	1.98	0.216	Subrahmanyan _et al._,1961
Casein	10	2.8±0.1	0.280	Present study
Casein	20	2.0+0.07	0.400	Chaves, 1961
Soybean + Casein (1:1)	10.7	1.84	0.197	Subrahmanyan _et al._,1961
Peanut + Soybean (1:1) + 2.0% Lysine + 3.0% Methionine	11.3	1.91	0.216	Subrahmanyan _et al._, 1961
Peanut + Bengal Gram + Sesame (5:3:2)	11.1	1.61	0.179	Subrahmanyan _et al._, 1961
Incaprina 8	25.2	2.71	0.683	Bressani, Scrimshaw, 1961
Modified 8	25.2	2.08	0.524	Bressani, Scrimshaw, 1961

TABLE 12.--Continued

Diet	Protein Content	PER, g gained/ g protein	Food Efficiency, g gained/ g food	Reference
SOY	15	1.7	0.255	Do-Quang-Oanh, 1963
SOY	15	2.0	0.300	Do-Quang-Oanh, 1963
SOY-ONLY	15	1.2	0.180	Do-Quang-Oanh, 1963
COD-SOY	15	2.4	0.36	Present study
	10	3.1	0.31	Present study
PAP	15	2.7	0.405	Do-Quang-Oanh, 1963
SOY-COD	15	2.4	0.36	Present study
	10	2.9	0.29	Present study
SOY-EGG	15	2.5	0.38	Present study
	10	3.3	0.33	Present study

exposure to high temperature and high humidity for a long time can be minimized. The proposed diets would probably have to be packaged in moisture proof containers in those high temperature, high humidity parts of the world where refrigeration is not available.

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